

Molecular cloning and expression analysis of FTZ-F1 in the GIFT tilapia, *Oreochromis niloticus*

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Abstract: The FTZ-F1 genes encode orphan receptors of the nuclear receptor superfamily and in mammals have been found to play important roles in the proper development of the adrenal-gonadal axis and sex-determination. We isolated the homologue of FTZ-F1 in genetically improved farmed tilapia (gFTZ-F1). The full-length cDNA was isolated from the ovary, which included an open reading frame encoding a predicted protein of 486 amino acids. Sequence, tissue distribution and phylogenetic analysis of the FTZ-F1 showed that the gFTZ-F1 belonged to SF-1/Ad4BP group and that gFTZ-F1 transcripts were only expressed in the gonads and kidney but not in other tissues. Likewise our data suggests that the gFTZ-F1 gene may share similar functions with other fish and mammalian counterparts, though further study is needed to make any definitive conclusions.

Keywords: GIFT tilapia; FTZ-F1; Cloning; Expression

Fushi tarazu factor-1 (FTZ-F1) is a member of the nuclear receptor superfamily originally described as a regulator of the *Drosophila* homeobox segmentation gene fushi tarazu (Lavorgna et al, 1991). FTZ-F1 homologues have since been recognized in numerous species and broadly divided into two subgroups of related genes with separate functions and expression patterns among higher vertebrates: LRH/FTF (liver receptor hormone/ α -fetoprotein transcription factor) and SF-1/Ad4BP (steroidogenic factor-1/adrenal 4 binding protein) (Ellinger-Ziegelbauer et al, 1994). The LRH/FTF genes are expressed mainly in the liver and are related to the regulation of the α -fetoprotein gene (Galameau et al, 1996) and the cholesterol metabolism (Nitta et al, 1999) while the SF-1/Ad4BP genes are involved in reproductive functions, regulating the transcription of many P450 enzymes acting in the steroidogenic pathways as well as by modulating the development of the hypothalamic pituitary adrenal and gonadal axis and the sex differentiation (Hammer & Ihgraham, 1999; Parker & Schimmer, 1997). The SF-1/Ad4BP genes also regulate the Mullerian inhibiting substance (MIS) transcription and promotion of testis development (Giuli et al, 1997; Shen et al, 1994).

In fish, several FTZ-F1 genes have been identified but some forms of FTZ-F1 homologues were not able to be classified in to either group (Von Hofsten et al, 2001), though the teleost FTZ-F1 may be a regulator in tissue differentiation and a factor in sexual maturation. We decided to study the gene in genetically improved farmed tilapia (GIFT), *Oreochromis niloticus*, a commercially important cultured freshwater fish in which males grow faster than females by 40%–50% (Holden & Reed, 1972). Regarding the potential role of FTZ-F1 homologues in the gonadal development and sex differentiation, it would be of significance to study the FTZ-F1 homologue in the

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GIFT tilapia. In this study, a homologue cDNA of FTZ-F1 was isolated from the ovary of adult GIFT tilapia in order to examine the expression pattern.

MATERIALS AND METHODS

Materials

Adult GIFT tilapia were maintained in a recirculating fresh water tanks at 26 ± 1 °C at the Experimental Base of Guangxi Fishery Institute, Nanning City, Guangxi Province, China.

For the cloning and measurement of FTZ-F1 mRNA in the GIFT tilapia, the gonads, brain, heart, liver, kidneys, spleen, intestinal, gills, muscle from adult female and male fish were quickly collected and snap frozen immediately in liquid nitrogen and stored at -80 °C until use.

RNA extraction and cDNA cloning

Total RNA extraction and reverse transcription were carried out as described previously (Cao et al, 2010). A pair of degenerate primers (P1: 5'-CGGAACAAGTTCGGCccnatnta-3' and P2: 5'-CCACGATGGAGAACAACGtytgrt-3') were designed according to the conserved sequences of the FTZ-F1 gene in other teleosts and used for isolation of a FTZ-F1 cDNA fragment of approximately 600 bp from the GIFT tilapia ovary. Amplification was performed at 94 °C for 2 min, 30 cycles of 94 °C for 30 s, 58 °C for 30 s and 72 °C for 1 min, and 72 °C for 10 min using a BIO-Rad gradient PCR thermal cycler. Then, 5'- and 3'-RACE were performed to obtain the 5' and 3' cDNA ends of FTZ-F1 using the 3'-Full RACE Core Set and 5'-Full RACE Kit (TaKaRa) according to the manufacturer's instructions. Two gene-specific primers were used for RACE: P3: 5'-GCTCCCCTGAGACTCTTACA-3'; P4: 5'-ATGCCGAAGTGAC CGTGGAA -3'.

The amplified products were separated and purified with agarose gel DNA purification Kit (TaKaRa). The purified fragments were then ligated into pMD 18-T Vector (TaKaRa), propagated in *E. coli* DH5 α . The recombinant plasmids were sequenced by Peking Audiocodes Biotech Co., Ltd..

Sequence analysis and alignment

The alignment of the FTZ-F1 protein's amino acid sequence was performed using the Clustal W algorithm 1.7 (European Bioinformatic Institute, Hinxton, United Kingdom) using default settings (Gap opening penalty 10, gap extension penalty 0.05, gap distance 8). A phylogenetic tree was generated with the neighbor-joining method using Mega 4.0. The sequences used for comparison and phylogenetic analysis and their GenBank accession numbers were as follows: *Acanthopagrus schlegelii* (sbFTZ-F1a: AAS75791), *Acanthopagrus*

schlegelii (sbFTZ-F1b: AAS75792), *Bos Taurus* (btAd4BP: BAA02764), *Cynoglossus semilaevis* (tsFTZ-F1: ABQ41307), *Clarias gariepinus* (acFTZ-F1a: AAG49004), *Clarias gariepinus* (acFTZ-F1b: AAG49005), *Carassius auratus* (Gftz-F1: AAM89250), *Danio danio* (zFF1a: AAK54449), *Danio danio* (zff1b: AAF43283), *Danio danio* (zff1c: AAK19303), *Danio danio* (zff1d: AAO59489), *Drosophila melanogaster* (dFTZ-F1: P33244), *Epinephelus coioides* (grouper FTZ-F1: AAQ72771), *Gallus gallus* (cFTF/LRH-1: BAA22838), *Gallus gallus* (cSF-1: BAA22839), *Homo sapiens* (hSF-1: AAB53105), *Homo sapiens* (hFTF: AAD03155), *Ictalurus punctatus* (ccNR5AA: AAY45704), *Mus musculus* (Mlrh-1: AAA39447), *Mus musculus* (Msf-1: AAB28338), *Oryzias latipes* (mdFTZ-F1: BAA32394), *Oncorhynchus mykiss* (rtFTZ-F1: AAW83490), *Oncorhynchus mykiss* (rtLRH-1: BAE71417), *Rana rugosa* (rrFTZ-F1a: BAA94077), *Rana rugosa* (rrSF-1: BAA36789) and *Trachemys scripta* (tuSF-1: AAD01975).

Real-time quantitative RT-PCR analysis of FTZ-F1 expression in the tissues

Total RNA was isolated from various tissues, cDNA was synthesized, and then real-time quantitative RT-PCR was conducted for analysis of FTZ-F1 expression using Roche lightcycler 480 Real-time PCR instrument (Roche, Switzerland) by means of the Real-time PCR Universal Reagent. A pair of gene specific primers (P5: 5'-ACACCCTTCCCACCATCTCTAA-3', P6: 5'-GGTGTAGTCGGGGCACTCG-3') were used to amplify a 194 bp FTZ-F1 cDNA fragment by touchdown real-time PCR. A 211 bp β -actin fragment was amplified as an internal control with a pair of β -actin primers (P7: 5'-GATGGTGGGTATGGGTCAGAA-3', P8: 5'-TTTGGGGTTCAGGGGAGC-3'). Relative fluorescence unit, calculated threshold cycle (Ct), and dissociation curve were monitored by the analysis software of the system. Triplicate assays per RNA sample were carried out to determine the average Ct values. Data of relative expression levels were analyzed according to previously described methods (Livak & Schmittgen, 2001). All the PCR products were electrophoresed on 1.0% agarose gel and visualized using ethidium bromide staining.

Statistical analysis

The averages of the data obtained were subjected to one-way ANOVA and Duncan's new multiple-range test using SPSS 15.0 (Chicago, Ill, USA). Significant differences among treatment averages were determined at a $P < 0.05$.

RESULTS

Isolation, characterization and phylogenetic analysis of the FTZ-F1 cDNA

After RT-PCR and subsequent 5' and 3'-RACE, a 1721 bp FTZ-F1cDNA was obtained (Figure 1). The

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                                CGC GGATCCACAGCCTACTG -260
ATG ATCACT CGATGG AAAACTCTG AGCTCTTG AATGGTCATCATCTACT ACA CTT TCTA -240
TTG GCC CTTCTGGCTCCTAA AGCATCCCGATATAGACAGCATCACCCGG AATTCTGT -180
GGCCTTCTCTTCTTTT TTTATT TTG TTC CCC TCTGACGCCCATGCAAGGCTGCTGCAACAC -120
CTACATCCA GGTTTT AATCAT CAGACAATCAT CACTG CCTGCCCTGCTGCT TGTGCT CAA -60
ATGTTGGGAGACAAAGGCTCACGGTGTGACTTTAAAGGTGATGGAGTACAC ATACGACGAG 60
M L G D K A H G V T L K V M E Y T Y D E 20

GACCTGGAAGAGCTGTGTCTGTGTGCGGAGACAAAGTGTCTGGATATCA CTACGGCTTG 120
D L E E L C P V C G D K V S G Y H Y G L 40

CTCACCTGTGAGAGCTGCAAGGGCTTCTTCAAGAGGACAGTGCAGAACAA CAAGAGGTAC 180
L T C E S C K G F F K R T V Q N N K R Y 60

ACGTGTGCGGAAAACAGGAGTGTATAATAGACAAGACCAGAGGAAAAG ATGTCCCTTC 240
T C A E N Q E C I I D K T Q R K R C P F 80

TGTCGGTTCCAGAAAGTGCCCTCAATGTTCGGGATGCGGCTGGAAGCGGTGCG TGCAGACCGC 300
C R F Q K C L N V G M R L E A V R A D R 100

ATGCGTGGGGGTAGGAATAAATTTGGCCCCATGTACAAGAGAGACAGGGC CTTAAACAG 360
M R G G R N K F G P M Y K R D R A L K Q 120

CAGAAGAAAGCTTTGATACGATCCAACGGTTTCAAGTTGGAGAGCACGGC TCCTCCACCA 420
Q K K A L I R S N G F K L E S T A P P P 140

GCCTCTCCTCTGCAGGCAGACTACGGCTTCACTGGCACCTTGCACACCCCT TCCCACCATC 480
A S P L Q A D Y G F T G T L H T L P T I 160

TCTAAAAGCTGCTCCCATCCACCCGAGCTCCATCACTCCACAGACTA CGAGGCTAAT 540
S K S L L P S T P S S I T P T D Y E A N 180

CTCTACGGACCCCGCTCTTTGGGTATGGCCATGCAGTCACAGTGCCCCCT CACCACCAG 600
L Y G P P S L G M A M Q S H V P L T T Q 200

TACCAGTACACAGCCTTTCCCGAAGGGCAATTAAGCGGAGTGCCCGCA CTACACCAGC 660
Y Q Y T A F P G R A I K A E C P D Y T S 220

TCCCTGAGACTCTTACAGGATACCCTTACCCAGACATGTACCCCTCAGC CTCGCCGCAA 720
S P E T L T G Y P Y P D M Y P S A S P Q 240

CCCCCAGCCTGCCCGCTGCTTCTGGAGCTGCTGCGCTGTGACCCGGA CGAGCTGGAC 780
P P S L P P L V L E L L R C D P D E L D 260

GTCCAGAACAAGATTGTGACTCACCTGCAACAGGAGCAAAACGGCCGGGG TCGGCTGGAA 840
V Q N K I V T H L Q Q E Q N G R G R L E 280

AAGCCAGCACCTTCAGCCTCATGTGTCGATGGCAGATCAAACGTTGTT CTCCATCGTG 900
K P S T F S L M C R M A D Q T L F S I V 300

GAGTGGGCTCGGAGCTGCATCTTCTTTAAGGAGCTGAGGGTGGGAGATCA AATGAAGCTG 960
E W A R S C I F F K E L R V G D Q M K L 320

CTTCATAACTGCTGGTCTGAACTGCTGGTCTGGATCACATTTTCAGACA AGTGCAACAC 1020
L H N C W S E L L V L D H I F R Q V Q H 340

GCAAAGGAAGACAGCATTTGCTGGTGACCGCCAGGAGGTGGAGCTGTC ATCCATCCTG 1080
A K E D S I L L V T G Q E V E L S S I L 360

TCTCAGGCTGAGGGGACACTCTCCAGTCTGGTCCAAAGAGGTGAGGAGCT GGCAGCGAGG 1140
S Q A E G T L S S L V Q R G Q E L A A R 380

CTGCGGGTGTGTCAGGTGACCGCAGAGAGATCGCCTGTCTGAAATTCCT CCTCCTTTTC 1200
L R V L Q V D R R E I A C L K F L L L F 400

AACCCAAATGTAAAATTGCTGGAGAACCAGGCGTTTGTGGAGGGCGTCCA GGAGCAGGTG 1260
N P N V K L L E N Q A F V E G V Q E Q V 420

AATGCTGCTCTGCTGGAGTACACCTGCTGCTACCTCAGTTCCAAGA GAAGTTCAGC 1320
N A A L L E Y T L S A Y P Q F Q E K F S 440

CAGCTGTTGGTACGGTTGCCGGAGCTGCGCTCCCTCAGCACGCAGGCCGA GGACTACCTA 1380
Q L L V R L P E L R S L S T Q A E D Y L 460

TGCTACATGCACGTGAGTGGAGAGGTGCCCTGCAACAACCTGCTGATCGA GATGCTGCAC 1440
C Y M H V S G E V P C N N L L I E M L H 480

GCTAAGAGGGCGTGTGTGTGA 1461
A K R A C V . 486

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Figure 1 cDNA nucleotide and predicted amino-acid sequences of the GIFT *Oreochromis niloticus* FTZ-F1

Nucleotide are indicated above and numbered to the right of each lane (upper row). The deduced amino acid sequence is shown below the nucleotide sequence. Amino acids are number to the right of each lane (lower row).

FTZ-F1 cDNA included an open reading frame, encoding a predicted 486 amino acid protein, and including the highly conserved DNA-binding and ligand-

bind regions (I,II and III, FTZ-F1 box) and the activation function-2 hexamer (Figure 2).The present sequence is available in GenBank database with the accession

[illegible]

Figure 2 Amino acid alignment of the GIFT tilapia FTZ-F1(gfFTZ-F1) with other species

Oryzias latipes (mdFTZ-F1: BAA32394), *Oncorhynchus mykiss* (rtFTZ-F1: AAW83490), *Gallus gallus* (cFTF/LRH-1: BAA22838), *Rana rugosa* (rtFTZ-F1: BAA94077), *Danio danio* (zFF1a: AAK54449), *Homo sapiens* (hSF-1: AAB53105), *Mus musculus* (Msf-1: AAB28338), *Epinephelus coioides* (grouper FTZ-F1: AAQ72771). The highly conserved regions of I, II, III, FTZ-F1 box and AF-2 hexamer are shown in gray fonts, black letters in black boxes, gray and boldface fonts, boldface fonts, and italics, respectively.

number JF742993.

The deduced amino acid sequence of the GIFT tilapia FTZ-F1 had 39%–94% of identity with that of other vertebrates. A higher level of identity was found when the GIFT tilapia FTZ-F1 was compared to the other teleosts FTZ-F1 proteins. The deduced amino acid sequence of the GIFT tilapia FTZ-F1 had 94%, 93%, 83%, 81% identity with that of the *O. latipes* FTZ-F1, *A. schlegelii* FTZ-F1b, *O. mykiss* FTZ-F1, *C. semilaevis* FTZ-F1, respectively; 68%, 68%, 65%, with that of the *T. scripta* SF-1, *G. gallus* SF-1, *R. rugosa* SF-1, respectively; 63%, 62% with that of *G. gallus* LRH-1, *M. musculus* LRH-1, respectively. The lowest sequence identity was with the *D. melanogaster* FTZ-F1. The GIFT tilapia FTZ-F1 was 68%, 67%, 63%, 46% sequence identity with *D. danio* fflb, fflb, ffla, fflc, respectively. The GIFT tilapia FTZ-F1 amino acid sequence and several published FTZ-F1 (as shown in Figure 3) in various vertebrate species were used to infer phylogenetic relationships. The phylogenetic tree shows that the GIFT tilapia clustered firmly with other teleosts FTZ-F1 (Figure 3).

Tissues expression of FTZ-F1

The FTZ-F1 mRNA expression in different tissues of the GIFT tilapia was analyzed using real-time PCR. The FTZ-F1 transcripts were highly expressed in testis and ovary and weakly in kidneys (Figure 4). No transcripts were found in brain, heart, liver, spleen, intestine, gills and muscle. The amounts of FTZ-F1 mRNA expression in the testis of adult male fish were approximately 3 fold of that in the male kidney (Figure 4) and the amounts of FTZ-F1 mRNA expression in the ovary of adult female fish were approximately 2.78 fold of that in the female kidney (Figure 4).

DISCUSSION

We successfully isolated a homologues FTZ-F1 from the GIFT tilapia ovary. Amino acid alignment analysis showed that the FTZ-F1 was characteristic of nuclear receptor superfamily, with highly conserved regions of I, II, III, FTZ-F1 box and AF-2 hexamer. Phylogenetic analysis of the GIFT tilapia FTZ-F1 showed that it clustered with other teleost FTZ-F1.

Likewise, the GIFT tilapia FTZ-F1 was expressed in the gonad and kidney, which suggested that the FTZ-F1 belonged to SF-1/Ad4BP (Hammer & Ihgraham, 1999; Parker & Schimmer, 1997). No transcripts of the GIFT tilapia FTZ-F1 were found in the liver, which implied that the FTZ-F1 did not belong to the LRH/FTF group (Lu et al, 2001).

There are a variety of evidences for FTZ-F1 activity in other fish. During embryogenesis of zebrafish, the expression of homologues FTZ-F1 was detected in the

pituitary, mandibular arch, pronephric duct, liver, rostral diencephalons, hindbrain, and pancreas (Chai & Chan, 2000; Von Hofsten et al, 2001), indicating that they might be involved in tissue differentiation. The tongue sole FTZ-F1 mRNA was detected in all stages from zygote to 25 days after hatching of the tongue sole, suggesting that FTZ-F1 might be involved in the organogenesis of the tongue sole embryo (Deng et al, 2008). The orange-spotted grouper *E. coioides* is a protogynous hermaphroditic fish, and the expression of FTZ-F1 in the gonad also decreased significantly in response to MT treatment (Zhang et al, 2007).

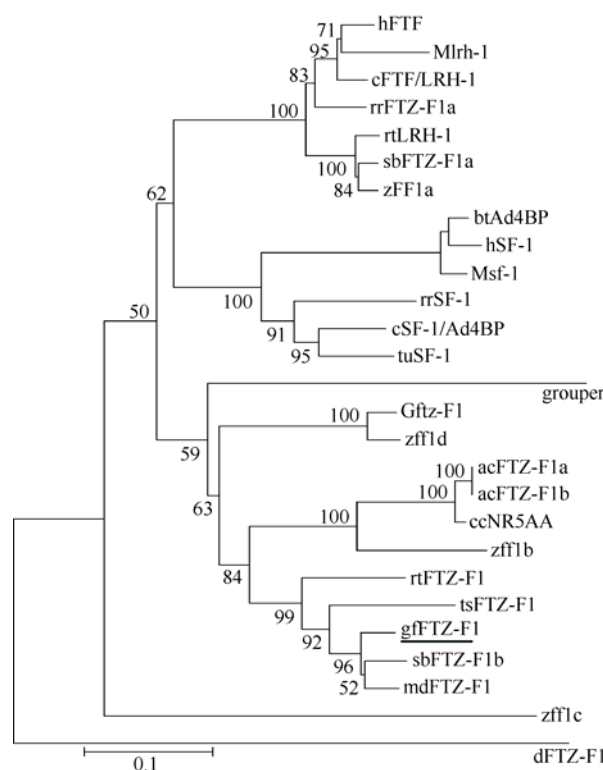


Figure 3 Neighbor joining tree of the GIFT Tilapia FTZ-F1 and FTZ-F1 from other organisms based on amino acid sequences

Distances are used to construct the phylogenetic tree and bootstrap values based on 1 000 resampling replicates. The bottom scale refers to percentage divergence (p-distance). *Acanthopagrus schlegelii* (sbFTZ-F1a: AAS75791), *Acanthopagrus schlegelii* (sbFTZ-F1b: AAS75792), *Bos taurus* (btAd4BP: BAA02764), *Cynoglossus semilaevis* (tsFTZ-F1: ABQ41307), *Clarias gariepinus* (acFTZ-F1a: AAG49004), *Clarias gariepinus* (acFTZ-F1b: AAG49005), *Carassius auratus* (Gftz-F1: AAM89250), *Danio danio* (zFF1a: AAK54449), *Danio danio* (zff1b: AAF43283), *Danio danio* (zff1c: AAK19303), *Danio danio* (zff1d: AAO59489), *Drosophila melanogaster* (dFTZ-F1: P33244), *Epinephelus coioides* (grouper FTZ-F1: AAQ72771), *Gallus gallus* (cFTF/LRH-1: BAA22838), *Gallus gallus* (cSF-1: BAA22839), *Homo sapiens* (hSF-1: AAB53105), *Homo sapiens* (hFTF: AAD03155), *Ictalurus punctatus* (ccNR5AA: AAY45704), *Mus musculus* (Mlrf-1: AAA39447), *Mus musculus* (Msf-1: AAB28338), *Oryzias latipes* (mdFTZ-F1: BAA32394), *Oncorhynchus mykiss* (rtFTZ-F1: AAW83490), *Oncorhynchus mykiss* (rtLRH-1: BAE71417), *Rana rugosa* (rrFTZ-F1a: BAA94077), *Rana rugosa* (rrSF-1: BAA36789), GIFT tilapia (gFTZ-F1: JF742993) and *Trachemys scripta* (tuSF-1: AAD01975).

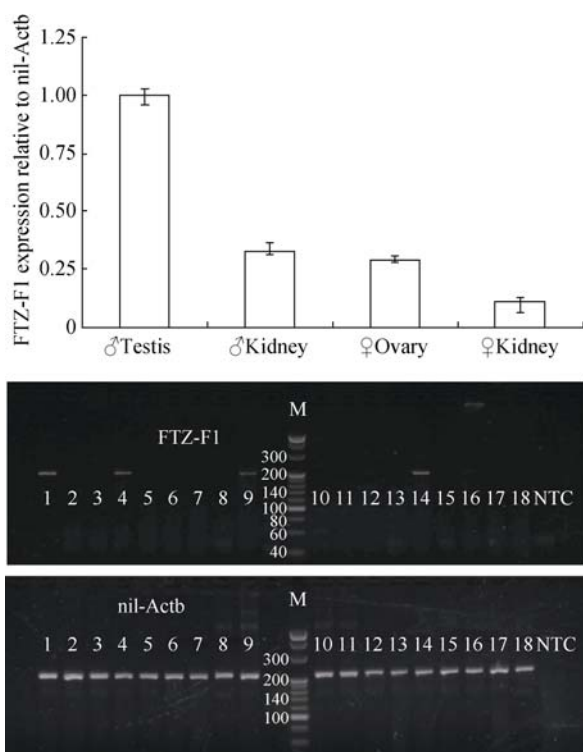


Figure 4 FTZ-F1 expression from various tissues of adult female and male, GIFT tilapia

1: ♂testis; 2: ♂brain; 3: ♂heart; 4: ♂kidney; 5: ♂spleen; 6: ♂intestine; 7: ♂gills; 8: ♂muscle; 9: ♀ovary; 10: ♀brain; 11: ♀muscle; 12: ♀heart; 13: ♀liver; 14: ♀kidney; 15: ♀spleen; 16: ♀intestine; 17: ♀gills; 18: ♂liver.

References

- Cao JL, Chen JJ, Wu TT, Gan X, Luo YJ. 2010. Molecular cloning and sexually dimorphic expression of DMRT4 gene in *Oreochromis aureus* [J]. *Mol Biol Rep*, **37**(6): 2781-2788.
- Chai C, Chan WK. 2000. Developmental expression of a novel Ftz-F1 homologue, *ff1b* (NR5A4), in the zebrafish *Danio rerio* [J]. *Mech Dev*, **91**(1-2): 421-426.
- Deng SP, Chen SL, Liu BW. 2008. Molecular cloning and expression analysis of FTZ-F1 in the half-smooth tongue-sole, *Cynoglossus semilaevis* [J]. *Zool Res*, **29**(6): 592-598.
- Ellinger-Ziegelbauer H, Hihi AK, Laudet V, Keller H, Wahli W, Dreyer C. 1994. FTZ-F1-related orphan receptors in *Xenopus laevis*: transcriptional regulators differentially expressed during early embryogenesis [J]. *Mol Cell Biol*, **14**(4): 2786-2797.
- Galameau L, Paré JF, Allard D, Hamel D, Levesque L, Tugwood JD, Green S, Bélanger L. 1996. The alpha1-fetoprotein locus is activated by a nuclear receptor of the Drosophila FTZ-F1 family [J]. *Mol Cell Biol*, **16**(7): 3853-3865.
- Giuli G, Shen WH, Ingraham HA. 1997. The nuclear receptor SF-1 mediates sexually dimorphic expression of Müllerian inhibiting substance, *in vivo* [J]. *Development*, **124**(9): 1799-1807.
- Hammer GD, Ingraham HA. 1999. Steroidogenic factor-1: Its role in

The Ad4BP/SF-1 increased in parallel with the onset of the female-phase and decreased as female became male in the serial sex changing goby, *Trimma okinawae* (Kobyashi et al, 2005). In *O. latipes*, the aromatase promoter contained potential binding site for steroidogenic factor 1(SF-1), which was involved in transcriptional regulation of P450 steroidogenic genes (cyp11A and B, steroid hydroxylases; aromatase) and in the formation of the gonads (Kuhl et al, 2005; Tanaka et al, 1995; Watanabe et al, 1999). It suggested the FTZ-F1 was involved in the regulation of sex reversal. Ultimately, however, whether the GIFT tilapia FTZ-F1 homologues may play roles similar to those of their mammalian counterparts needs to be tested by more functional studies.

CONCLUSIONS

FTZ-F1 (gfFTZ-F1) full-length cDNA from the ovary in GIFT tilapia included an open reading frame encoding a predicted 486 amino acids. Sequence, tissue distribution and phylogenic analysis of the FTZ-F1 showed that the gfFTZ-F1 belonged to SF-1/Ad4BP group as the gfFTZ-F1 transcripts were only expressed in the gonads and kidney, not in other tissues. The amounts of gfFTZ-F1 mRNA expression in the testis were the highest, while the amounts in the kidney of the adult female GIFT tilapia were the lowest.

endocrine organ development and differentiation [J]. *Front Neuroendocrinol*, **20**(3): 199-223.

Holden M, Reed W. 1972. West African Freshwater Fish [M]. London: Longman Group Ltd.

Kobyashi Y, Sunobe T, Kobayashi T, Nakamura M, Suzuki N, Nagahama Y. 2005. Molecular cloning and expression of *Ad4BP/SF-1* in the serial sex changing gobiid fish, *Trimma okinawae* [J]. *Biochem Biophys Res Commun*, **332**(4): 1073-1080.

Kuhl AJ, Manning S, Brouwer M. 2005. Brain aromatase in Japanese medaka (*Oryzias latipes*): Molecular characterization and role in xenoestrogen-induced sex reversal [J]. *J Steroid Biochem Mol Biol*, **96**(1): 67-77.

Lavorgna G, Ueda H, Clos J, Wu C. 1991. FTZ-F1, a steroid hormone receptor-like protein implicated in the activation of fushi tarazu [J]. *Science*, **252**(5007): 848-851.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method [J]. *Methods*, **25**(4): 402-408.

Lu TT, Repa JJ, Mangelsdorf DJ. 2001. Orphan nuclear receptors as eLixiRs and FiXeRs of sterol metabolism [J]. *J Biol Chem*, **276**(41): 37735-37738.

- Nitta M, Ku S, Brown C, Okamoto A Y, Shan B. 1999. CPF: An orphan nuclear receptor that regulates liver-specific expression of the human cholesterol 7 α -hydroxylase gene [J]. *Proc Nat Acad Sci USA*, **96**(12): 6660-6665.
- Parker KL, Schimmer BP. 1997. Steroidogenic factor 1: a key determinant of endocrine development and function [J]. *Endocr Rev*, **18**(3): 361-377.
- Shen P, Campagnoni CW, Kampf K, Schlinger BA, Arnold AP, Campagnoni AT. 1994. Isolation and characterization of a zebra finch aromatase cDNA: in situ hybridization reveals high aromatase expression in brain [J]. *Mol Brain Res*, **24**(1-4): 227-237.
- Tanaka M, Fukada S, Matsuyama M, Nagahama Y. 1995. Structure and promoter analysis of the cytochrome P-450 aromatase gene of the teleost fish, medaka (*Oryzias latipes*) [J]. *J Biochem*, **117**(4): 719-725.
- Von Hofsten J, Jones I, Karlsson J, Oisson PE. 2001. Developmental expression patterns of FTZ-F1 homologues in zebrafish (*Danio rerio*) [J]. *Gen Comp Endocrinol*, **121**(2): 146-155.
- Watanabe M, Tanaka M, Kobayashi D, Yoshiura Y, Oba Y, Nagahama Y. 1999. Medaka (*Oryzias latipes*) FTZ-F1 potentially regulates the transcription of P-450 aromatase in ovarian follicles: cDNA cloning and functional characterization [J]. *Mol Cell Endocrinol*, **149**(1-2): 221-228.
- Zhang WM, Zhang Y, Zhang LH, Zhao HH, Li X, Huang H, Lin HR. 2007. The mRNA expression of P450 aromatase, gonadotropin β -subunits and FTZ-F1 in the orange-spotted grouper (*Epinephelus coioides*) during 17 α -methyltestosterone-induced precocious sex change [J]. *Mol Reprod Dev*, **74**(6): 665-673.